



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 257/14, A61K 31/155 C07C 257/16, 323/58	A1	(11) International Publication Number: WO 93/13055 (43) International Publication Date: 8 July 1993 (08.07.93)
(21) International Application Number: PCT/GB92/02387 (22) International Filing Date: 23 December 1992 (23.12.92) (30) Priority data: 9127376.3 24 December 1991 (24.12.91) GB (71) Applicant (for all designated States except US): THE WELL-COME FOUNDATION LIMITED [GB/GB]; Unicorn House, 160 Euston Road, London NW1 2BP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : BEAMS, Richard, Mansfield [GB/GB]; HODSON, Harold, Francis [GB/GB]; PALMER, Richard, Michael, John [GB/GB]; Langley Court, Beckenham, Kent BR3 3BS (GB).		(74) Agent: ROLLINS, Anthony, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB). (81) Designated States: AU, CA, CS, FI, HU, JP, KR, NO, NZ, PL, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: AMIDINO DERIVATIVES AND THEIR USE AS NITRIC OXIDE SYNTHASE INHIBITORS <div style="text-align: center; margin: 20px 0;"> $\begin{array}{c} \text{R}^1 \\ \\ \text{HN}=\text{C}-\text{NH}-\text{Q}-\text{CH}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array} \quad (I)$ </div> (57) Abstract <p>Amidino derivatives of formula (I) and salts, and pharmaceutically acceptable esters and amides thereof, in which: R¹ is a C₁₋₆ straight or branched chain alkyl group, a C₂₋₆alkenyl group, a C₂₋₆alkynyl group, a C₃₋₆cycloalkyl group or a C₃₋₆cycloalkylC₁₋₆alkyl group; Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted by one or more C₁₋₃alkyl groups; a group of formula -(CH₂)_pX(CH₂)_q- where p is 2 or 3, q is 1 or 2 and X is S(O)_x where x is 0, 1 or 2, O or NR² where R² is H or C₁₋₆alkyl; or a group of formula -(CH₂)_rA(CH₂)_s- where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents such as C₁₋₆alkyl, C₁₋₆alkoxy, hydroxy, halo, nitro, cyano, trifluoroC₁₋₆alkyl, amino, C₁₋₆alkylamino or diC₁₋₆alkylamino; and pharmaceutical formulations containing them are described for use in medicine, novel compounds of formula (I) and the preparation of such novel compounds are also disclosed.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

Amidino derivatives and their use as nitric oxide synthase inhibitors

The present invention relates to amidino derivatives,
5 to methods for their manufacture, to pharmaceutical
compositions containing them and to their use in therapy,
in particular their use as nitric oxide synthase
inhibitors.

It has been known since the early 1980's that the
10 vascular relaxation brought about by acetylcholine is
dependent on the presence of the endothelium and this
activity was ascribed to a labile humoral factor termed
endothelium-derived relaxing factor (EDRF). The activity
15 of nitric oxide (NO) as a vasodilator has been known for
well over 100 years and NO is the active component of
amyl nitrite, glyceryl trinitrite and other
nitrovasodilators. The recent identification of EDRF as NO
has coincided with the discovery of a biochemical pathway
20 by which NO is synthesised from the amino acid L-arginine
by the enzyme NO synthase.

NO is the endogenous stimulator of the soluble
guanylate cyclase and is involved in a number of biological
actions in addition to endothelium-dependent relaxation
including cytotoxicity of phagocytic cells and cell-to-cell
25 communication in the central nervous system (see Moncada et
al, *Biochemical Pharmacology*, 38, 1709-1715 (1989) and
Moncada et al, *Pharmacological Reviews*, 43, 109-142
(1991)). It is now thought that excess NO production may
be involved in a number of conditions, particularly
30 conditions which involve systemic hypotension such as toxic
shock and therapy with certain cytokines.

The synthesis of NO from L-arginine can be inhibited
by the L-arginine analogue L-N-monomethyl-arginine (L-NMMA)
and the therapeutic use of L-NMMA for the treatment of
35 toxic shock and other types of systemic hypotension has
been proposed (WO 91/04024 and GB-A-2240041). The
therapeutic use of certain other NO synthase inhibitors

apart from L-NMMA for the same purpose has also been proposed in WO 91/04024 and in EP-A-0446699.

It has recently become apparent that there are at least two types of NO synthase as follows:

5 (i) a constitutive, Ca^{++} /calmodulin dependent enzyme that releases NO for response to receptor or physical stimulation.

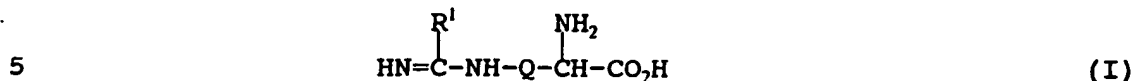
10 (ii) a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed this inducible NO synthase synthesises NO for long periods.

15 The NO released by the constitutive enzyme acts as a transduction mechanism underlying several physiological responses. The NO produced by the inducible enzyme is as a cytotoxic molecule for tumour cells and invading microorganisms. It also appears that the adverse effects of excess NO production, in particular pathological vasodilation and tissue damage, may result largely from the effects of NO synthesised by the inducible NO synthase.

20 The NO synthase inhibitors proposed for therapeutic use so far, and in particular L-NMMA, are non-selective in that they inhibit both the constitutive and the inducible NO synthase. Use of such a non-selective NO synthase inhibitor requires that great care be taken in order to avoid the potentially serious consequences of over-inhibition of the constitutive NO-synthase including hypotension and possible thrombosis and tissue damage. In particular, in the case of the therapeutic use of L-NMMA for the treatment of toxic shock it has been recommended that the patient must be subject to continuous blood pressure monitoring throughout the treatment. Thus, whilst non-selective NO synthase inhibitors have therapeutic utility provided that appropriate precautions are taken, NO synthase inhibitors which are selective in the sense that they inhibit the inducible NO synthase to a considerably greater extent than the constitutive NO synthase would be

of even greater therapeutic benefit and much easier to use.

The present invention concerns amidino derivatives of the formula (I)



and salts, and pharmaceutically acceptable esters and amides thereof, in which:

R^1 is a C_{1-6} straight or branched chain alkyl group, a C_{2-6} alkenyl group, a C_{2-6} alkynyl group, a C_{3-6} cycloalkyl group or a C_{3-6} cycloalkyl C_{1-6} alkyl group;

Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl groups;

a group of formula $-(\text{CH}_2)_p\text{X}(\text{CH}_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is $\text{S}(\text{O})_x$ where x is 0, 1 or 2, 0 or NR^2 where R^2 is H or C_{1-6} alkyl; or

a group of formula $-(\text{CH}_2)_r\text{A}(\text{CH}_2)_s-$ where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents such as C_{1-6} alkyl, C_{1-6} alkoxy, hydroxy, halo, nitro, cyano, trifluoro C_{1-6} alkyl, amino, C_{1-6} alkylamino or di C_{1-6} alkylamino.

The compounds of formula (I) may include a number of asymmetric centres in the molecule depending on the precise meaning of the various groups and formula (I) is intended to include all possible isomers. When the group Q is an alkenyl group such as $-\text{CH}_2\text{CH}=\text{CHCH}_2-$, both the E and Z isomers are included. The compounds of formula (I)

all include an asymmetric centre in the group $\begin{array}{c} \text{NH}_2 \\ | \\ -\text{CH}-\text{CO}_2\text{H} \end{array}$ and although the natural L or (S) chirality of arginine is preferred, it is again intended that the formula should include all possible isomers.

The group A as a carbocyclic or heterocyclic ring may be saturated, may include ethylenic unsaturation or may be aromatic. In the case of a heterocyclic ring the ring may

contain one or more heteroatoms which will generally be selected from N, O and S.

Pharmaceutically acceptable esters and amides of the compounds of formula (I) may have the $-CO_2H$ end group replaced by $-CO_2R^3$ where R^3 is for example C_{1-6} alkyl, aryl or aryl C_{1-3} alkyl or $-COR^4$ where R^4 is the residue of a suitable natural or synthetic amino acid.

According to one aspect, the present invention provides the use of a compound of formula (I) as defined above or a pharmaceutically acceptable salt, ester or amide thereof for use in the manufacture of a medicament for the treatment of a condition where there is an advantage in inhibiting NO production from L-arginine by the action of NO synthase.

According to a further aspect, the present invention provides a method for the treatment of a human or animal subject suffering from a condition where there is an advantage in inhibiting NO production from L-arginine by the action of NO synthase which comprises administering to the subject an effective amount to relieve said condition of a compound of formula (I) as defined above or a pharmaceutically acceptable salt, ester or amides thereof.

The activity of a compound as an inhibitor of the enzyme NO synthase can be determined by an assay in which a macrophage cell line (J774) is induced to express NO synthase with lipopolysaccharide (LPS) and interferon gamma and NO generated is measured by its reaction with haemoglobin or as nitrite. This assay has the advantage of convenience but does not distinguish between selective and non-selective NO synthase inhibitors. Selectivity can be determined by use of an assay based on rat aortic rings in vitro. The basal tone of the tissue is used as an indication of the activity of the constitutive NO synthase whereas LPS-induced loss of tone mediated by NO is used as an indication of the activity of the inducible enzyme in the vascular smooth muscle cells. Selectivity can also be demonstrated by use of an assay based on the measurement of

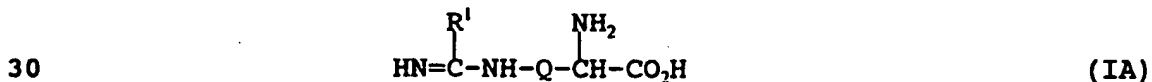
rat blood pressure (constitutive enzyme) and rat blood pressure in LPS treated rats (inducible enzyme). Compounds of formula (I) show activity as inhibitors of NO synthase in the J774 cell line and a number of compounds have also
 5 been demonstrated to be selective inhibitors in the rat aortic ring, in particular they have been demonstrated to be some 10 to 40 times more potent as inhibitors of the inducible NO synthase than the constitutive NO synthase.

Conditions in which there is an advantage in
 10 inhibiting NO production from L-arginine include systemic hypotension associated with septic and/or toxic shock induced by a wide variety of agents; therapy with cytokines such as TNF, IL-1 and IL-2; and as an adjuvant to short term immunosuppression in transplant therapy.

15 There is also a growing body of evidence for the existence of a third NO synthase enzyme which may be involved in the degeneration of cartilage which takes place in certain conditions such as arthritis and it is also known that NO synthesis is increased in rheumatoid
 20 arthritis. Accordingly further conditions in which there is an advantage in inhibiting NO production from L-arginine include autoimmune and/or inflammatory conditions affecting the joints, for example arthritis.

As used herein, reference to "treatment" of a patient
 25 is intended to include prophylaxis.

Certain compounds of formula (I) are novel and according to a further aspect the invention provides amidino derivatives of the formula (IA)



and salts, and pharmaceutically acceptable esters and amides thereof, in which:

R¹ is a C₁₋₆ straight or branched chain alkyl group, a C₂₋₆alkenyl group, a C₂₋₆alkynyl group, a C₃₋₆cycloalkyl group
 35 or a C₃₋₆cycloalkylC₁₋₆alkyl group;

Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted

by one or more C₁₋₃alkyl groups;

a group of formula $-(CH_2)_pX(CH_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is S(O)_x where x is 0, 1 or 2, O or NR² where R² is H or C₁₋₆alkyl; or

5 a group of formula $-(CH_2)_rA(CH_2)_s-$ where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents such as C₁₋₆alkyl, C₁₋₆alkoxy, hydroxy, halo, nitro, cyano, trifluoroc₁₋₆alkyl, amino, C₁₋₆alkylamino or diC₁₋₆alkylamino;

10 with the proviso that Q is neither CH₂CH₂CH₂CH₂ nor CH₂CH₂CH₂ when R¹ is methyl.

According to another aspect, the present invention also provides compounds of formula (IA) as defined above and pharmaceutically acceptable salts, esters and amides thereof for use in therapy, in particular the therapy of conditions where there is an advantage in inhibiting NO production from L-arginine by the action of NO synthase.

15 Specific examples of the group R¹ include methyl, ethyl, n-propyl, isopropyl, cyclopropyl, propenyl, n-butyl, t-butyl, cyclobutyl and cyclopropylmethyl.

Specific examples of the group A as a carbocyclic or heterocyclic ring include cyclohexyl, cyclopentyl cyclobutyl, cyclopropyl, cyclohexenyl, cyclopentenyl, cyclobutenyl, phenyl, pyridyl, piperidinyl, pyrrolyl, pyrrolidinyl, furanyl, tetrahydrofuranyl, thiazolyl, imidazolyl and oxazolyl. In each case the ring may optionally be substituted by one or more suitable substituents such as the substituents mentioned above.

25 Particular examples of Q as an alkylene, alkenylene or alkynylene group includes groups of the formulae

$-(CH_2)_n-$ where n is 3 to 5;

$-(CH_2)_vCH=CH(CH_2)_w$; and

$-(CH_2)_vC\equiv C(CH_2)_w$; where in each case v is 0 to 3 and w

35 is 0 to 3 with the proviso that the sum total of v + w is 2 to 4.

A preferred group of compounds of formula (I) are compounds in which:

R¹ is methyl;

Q is $-(CH_2)_n-$ where n is 3 to 5, more preferably 3 or 4;

5 $-CH_2CH=CHCH_2-$;

$-(CH_2)_pX(CH_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is $S(O)_x$ where x is 0, 1 or 2, more preferably 0, 0 or NR^2 where R² is H or C₁₋₆alkyl, more preferably C₁₋₃alkyl; or

10 $-(CH_2)_rA(CH_2)_s-$ where r is 1 or 2, s is 1 or 2 and A is a 6 membered carbocyclic or heterocyclic ring, more preferably one of the specific rings referred to above, most preferably cyclobutyl, phenyl or pyridyl;

with the proviso that the group $-CH-\overset{NH_2}{\overset{|}{CO_2H}}$ has the natural L or (S) chirality;
15 and pharmaceutically acceptable salts, esters and amides thereof.

A particularly preferred group of compounds of formula (I) are compounds in which:

20 R¹ is methyl;

Q represents $-(CH_2)_n-$ where n is 3 or 4;

$-CH_2CH=CHCH_2-$;

$-(CH_2)_2SCH_2-$; or

$-CH_2ACH_2-$ where A is cyclopropyl;

25 and pharmaceutically acceptable salts, esters and amides thereof.

Specific preferred compounds of formula (I) are:

(S)-N⁵-(1-iminoethyl)ornithine;

(S)-N⁶-(1-iminoethyl)lysine;

30 (±)-E-2-amino-6-(1-iminoethylamino)-hex-4-enoic acid;

and

(S)-S-2-(1-iminoethylamino)ethylcysteine

and pharmaceutically acceptable salts, esters and amides thereof.

35 The present invention includes compounds of formula (I) in the form of salts, in particular acid addition

salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Thus, preferred salts include those formed from hydrochloric, hydrobromic, sulphuric, citric, tartaric, phosphoric, lactic, pyruvic, acetic, succinic, oxalic, fumaric, maleic, oxaloacetic, methanesulphonic, ethanesulphonic, p-toluenesulphonic, benzenesulphonic and isethionic acids. Salts of the compounds of formula (I) can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

Whilst it may be possible for the compounds of formula (I) to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising a compound of formula (I) or (IA) or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof ("active ingredient")

with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided
5 solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a
10 predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus,
15 electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing
20 form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The
25 tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which
30 may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations
35 may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the

addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges comprising the active ingredient in a flavoured basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The compounds of the invention may be administered orally or via injection at a dose of from 0.1 to 500mg/kg per day. The dose range for adult humans is generally from 5mg to 35g/day and preferably 5mg to 2g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5mg to 500mg, usually around 10mg to 200mg.

The compounds of formula (I) are preferably administered orally or by injection (intravenous or subcutaneous). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However the dose employed will depend on a

number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also the route of administration may vary depending on the condition and its severity.

5 Compounds of formula (I) can be prepared by reaction of an amino acid of formula (II)



or a protected derivative thereof, with a compound of
10 formula (III)



where L is a leaving group and R¹ and Q are as defined above, followed by removal of any protecting groups
15 present.

Suitable leaving groups L include -OR^s and -SR^s where R^s is a lower alkyl group, e.g. C₁₋₄alkyl, preferably methyl or ethyl.

The compound of formula (II) will generally be used in
20 a form in which the amino acid functionality is protected by suitable protecting groups and in this connection reference can be made to T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 2nd Edition, John Wiley and Sons Inc., 1991. The protecting groups can then
25 be removed in conventional manner (*loc. cit.*) as the final stage of the process to yield the compound of formula (I). For example the amino acid functionality can be protected as the copper salt with deprotection taking place on an ion exchange column which is employed to remove inorganic ions
30 from the reaction mixture.

The compounds of formula (III) can be used in the form of the free base or as an acid addition salt, e.g. the hydrochloride or hydroiodide salt.

The reaction is generally carried out in a suitable
35 solvent in the presence of base, e.g. an alkali metal hydroxide such as sodium hydroxide, preferably at a pH of

about 9 to 11 and generally at a temperature from 0°C up to the reflux temperature of the solvent, preferably 0°C to 50°C. The preferred solvent is water although the reaction may also be carried out in a polar solvent such as a lower alcohol, e.g. methanol or ethanol, or an amide, e.g. dimethylformamide, either alone or in admixture with water, and this may be advantageous in certain circumstances.

The compounds of formula (II) are in general known compounds which can be converted into appropriate protected derivatives in known manner. The imidates and thioimides of formula (III) (L is -OR⁵ and -SR⁵ respectively) are also in general known compounds and the reaction of such compounds with a primary amine is discussed for example in The Chemistry of Amidines and Imidates, Vol. 2, Eds. Saul Patai and Zvi Rappaport, John Wiley and Sons Inc., 1991.

The invention is illustrated by the following Examples.

EXAMPLES

Example 1

(S)-N⁵-(1-Iminoethyl)ornithine hydrochloride

L-Ornithine hydrochloride (10.1g, 60mmol) was dissolved in hot water (10ml) and the solution was stirred at 100° during the portionwise addition of copper carbonate (7g) over 15 minutes. The dark blue solution was cooled to room temperature, filtered, and the residue was washed with water (20ml).

The combined filtrate and washings were stirred at 5° during the portionwise addition of ethyl acetimidate hydrochloride (11.1g, 90mmol); throughout the addition the solution was maintained at a pH of 10.5 by simultaneous addition of 2N aqueous sodium hydroxide. The solution was then allowed to stand at room temperature for one hour, acidified with 2N aqueous hydrochloride to pH3 and applied to a column (150 ml) of cation exchange resin Bio-Rad AG 50W-X8 (hydrogen form). The column was washed with water

until the eluate was neutral and then eluted with 0.5N aqueous ammonia. The ninhydrin positive fractions were combined and evaporated under reduced pressure at 40°C for a short time to remove ammonia. The solution was then
5 carefully acidified to pH4 with 2N aqueous hydrochloric acid and evaporated to dryness under reduced pressure.

The residue was crystallised by dissolving in a little water, adding a large volume of ethanol and allowing the mixture to stand at 4° for a few hours. The precipitate
10 was filtered, washed with ethanol, and dried in a desiccator under reduced pressure to give the title compound as a colourless crystalline solid mp. 226-228°.

Example 2

(S)-N⁶-(1-Iminoethyl) lysine hydrochloride

Essentially by the methods of Example 1 L-lysine hydrochloride (7.3g, 40mmol) was converted to the copper complex which was reacted with ethyl acetimidate hydrochloride (7.5g, 60mmol). The title compound, isolated
20 as described in Example 1, was obtained as a hygroscopic amorphous powder.

Example 3

(±)-E-2-Amino-6-(1-Iminoethylamino)-hex-4-enoic acid hydrochloride

The methods of Example 1 were used to convert E-4,5-dehydrolysine hydrochloride to the title compound which was obtained in an amorphous state, homogenous by t.l.c. in a mixture of acetonitrile/water/acetic acid
30 (5:3:2), and with n.m.r. spectrum (D₂O) and mass spectrum consistent with the proposed structure.

Example 4

(S)-S-2-(1-Iminoethylamino)ethylcysteine hydrochloride

Using the methods of Example 1
35 (S)-S-2-(1-aminoethylcysteine hydrochloride (2g, 10mmol) was converted to the copper complex, and reacted with ethyl

acetimidate hydrochloride (1.88g, 15mmol). The product was isolated as described in Example 1 to give the title compound as a pale yellow amorphous solid, homogeneous by t.l.c. in a mixture of acetonitrile/water/acetic acid (5:3:2), and with n.m.r. spectrum (D₂O) and mass spectrum consistent with the proposed structure.

The compounds of Examples 5 to 11 were prepared, essentially by the methods of Example 1, by reaction of the copper complex of the appropriate amino acid of formula (II) with the relevant ethyl alkanimidate hydrochloride.

Example 5

(S)-N⁶-(1-Iminopropyl)lysine hydrochloride

The title compound was isolated as a hygroscopic amorphous white powder, homogeneous by t.l.c. in a mixture of acetonitrile/water/acetic acid (5:3:2).
Mass spectrum: (M+1)⁺, 202.0.

Example 6

(S)-N⁶-(α -Iminocyclopropylmethyl)lysine hydrochloride

The title compound was isolated as a hygroscopic amorphous white powder, homogeneous by t.l.c.
Mass spectrum: (M+1)⁺, 214.0.

Example 7

(\pm)-2-Amino-7(1-iminoethylamino)-heptanoic acid hydrochloride

The title compound was obtained as amorphous material, homogeneous by t.l.c., in a mixture of acetonitrile/water/acetic acid (5:3:2).

Mass spectrum: (M+1)⁺, 202.0.

¹H-NMR (200 M-Hz, D₂O): 1.22-1.43 (m, 2CH₂), 1.50-1.70 (m CH₂), 1.75-1.90 (m, CH₂), 2.1 (s, CH₃), 3.10-3.20 (t, CH₂), 3.60-3.72 (t, CH).

Example 8(±)-2-2-Amino-6-(1-iminoethylamino)-hex-4-enoic acid hydrochloride

The title compound crystallised from a mixture of ethanol and water to give colourless crystals, mp. 223-225°, homogeneous by t.l.c. in a mixture of methanol/.880 ammonia (7:3).

Mass spectrum: (M+1)⁺, 186.0. ²H-NMR (200 M-Hz, D₂O): 2.22 (s, CH₃), 2.66-2.76 (m, CH₂), 3.80-3.90 (t, CH) 3.80-4.00 (d, CH₂), 5.70-5.80 (m, 2 CH).

Example 9(S)-N⁵-(1-Iminoprop-1-yl)ornithine hydrochloride

The title compound was obtained as a pale yellow amorphous solid, homogeneous by t.l.c. in a mixture of acetonitrile/water/acetic acid (5:3:2).

Mass spectrum: (M+1)⁺, 188.0. ²H-NMR (200 M-Hz, D₂O): 1.05-1.08, (t, CH₃), 1.55-1.90 (m, 2CH₂), 2.30-2.45 (q, CH₂), 3.13-3.28 (t, CH₂), 3.62-3.72 (t, CH).

Example 10(S)-N⁵-(1-Iminobut-2-yl)ornithine hydrochloride

The title compound crystallised from ethanol containing a trace of water to give a crystalline solid mp. 202°, homogeneous by t.l.c., in a mixture of acetonitrile/water/acetic acid (5:3:2). ¹H-NMR (200 M-Hz, D₂O): 1.21-1.29, (d, 2CH₃), 1.61-1.85 (m, CH₂), 1.82-2.01 (m, CH₂) 2.65-2.88, (s, CH), 3.25-3.39 (t, CH₂), 3.74-3.84 (t, CH).

Example 11(S)-N⁵-(α-Iminocyclopropylmethyl)ornithine hydrochloride

The title compound crystallised from a mixture of ethanol and water to give a colourless crystalline solid mp. 2.75-2.77°, homogeneous by t.l.c., in a mixture of acetonitrile/water/acetic acid (5:3:2).

Mass spectrum: $(M+1)^+$, 200. $^1\text{H-NMR}$ (200 M- H_2 , D_2O): 1.0-1.30 (m, 2CH_2), 1.60-2.05 (m, 2CH_2 , CH), 3.25-3.40 (t, CH_2), 3.70-3.85 (t, CH).

5 Biological Data

The activity of certain compounds of formula (I) as NO synthase inhibitors has been determined in the following assays:

10 Constitutive NO synthase - method of Rees et al, Br. J. Pharmacol., 101, 746-752 (1990)

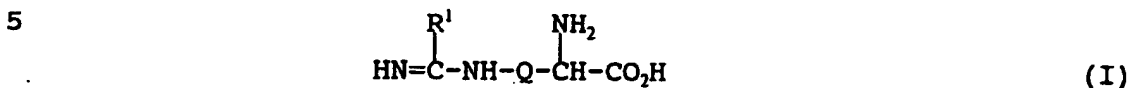
Inducible NO synthase - method essentially according to Rees et al, Biochem. Biophys. Res. Comm., 173, 541-547 (1990).

15 The results were as follows:

Compound of Example No.	NO Synthase Activity		EC ₅₀ ratio
	Constitutive EC ₅₀ μM	Inducible EC ₅₀ μM	
1	5.1	0.14	36.4
2	6.9	0.16	43.1
3	3.1	0.32	9.7
4	4.3	0.13	33.4

CLAIMS:

1. The use of amidino derivatives of the formula (I)



and salts, and pharmaceutically acceptable esters and amides thereof, in which:

10 R^1 is a C_{1-6} straight or branched chain alkyl group, a C_{2-6} alkenyl group, a C_{2-6} alkynyl group, a C_{3-6} cycloalkyl group or a C_{3-6} cycloalkyl C_{1-6} alkyl group;

Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl groups;

15 a group of formula $-(\text{CH}_2)_p\text{X}(\text{CH}_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is $\text{S}(\text{O})_x$ where x is 0, 1 or 2, 0 or NR^2 where R^2 is H or C_{1-6} alkyl; or

20 a group of formula $-(\text{CH}_2)_r\text{A}(\text{CH}_2)_s-$ where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents such as C_{1-6} alkyl, C_{1-6} alkoxy, hydroxy, halo, nitro, cyano, trifluoroc C_{1-6} alkyl, amino, C_{1-6} alkylamino or di C_{1-6} alkylamino; for use in the manufacture of a medicament for the treatment of a

25 condition where there is an advantage in inhibiting NO production from L-arginine by the action of NO synthase.

30 2. A method for the treatment of a human or animal subject suffering from a condition where there is an advantage in inhibiting NO production from L-arginine by the action of NO synthase which comprises administering to the subject an effective amount to relieve said condition of a compound of formula (I) as defined above or a pharmaceutically acceptable salt, ester or amide thereof.

35

3. An amidino derivative of the formula (IA)



and salts, and pharmaceutically acceptable esters and amides thereof, in which:

5 R^1 is a C_{1-6} straight or branched chain alkyl group, a C_{2-6} alkenyl group, a C_{2-6} alkynyl group, a C_{3-6} cycloalkyl group or a C_{3-6} cycloalkyl C_{1-6} alkyl group;

Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl groups;

10 a group of formula $-(\text{CH}_2)_p\text{X}(\text{CH}_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is $\text{S}(\text{O})_x$ where x is 0, 1 or 2, 0 or NR^2 where R^2 is H or C_{1-6} alkyl; or

15 a group of formula $-(\text{CH}_2)_r\text{A}(\text{CH}_2)_s-$ where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents; with the proviso that Q is neither $\text{CH}_2\text{CH}_2\text{CH}_2$ nor $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ when R^1 is methyl.

20 4. A compound of formula (IA) according to Claim 3 wherein R^1 is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, propenyl, n-butyl, t-butyl, cyclobutyl or cyclopropylmethyl;

25 A is cyclohexyl, cyclopentyl cyclobutyl, cyclopropyl, cyclohexenyl, cyclopentenyl, cyclobutenyl, phenyl, pyridyl, piperidinyl, pyrrolyl, pyrrolidinyl, furanyl, tetrahydrofuranyl, thiazolyl, imidazolyl and oxazolyl, each optionally substituted by one or more suitable substituents;

30 Q is a group $-(\text{CH}_2)_n-$ where n is 3 to 5;

$-(\text{CH}_2)_v\text{CH}=\text{CH}(\text{CH}_2)_w$; and

$-(\text{CH}_2)_v\text{C}\equiv\text{C}(\text{CH}_2)_w$; where in each case v is 0 to 3 and w is 0 to 3 with the proviso that the sum total of $v + w$ is 2 to 4.

35

5. A compound according to either Claims 3 or 4 wherein

the optional substituents on the group A are selected from C₁₋₆alkyl, C₁₋₆alkoxy, hydroxy, halo, nitro, cyano, trifluoroc₁₋₆alkyl, amino, C₁₋₆alkylamino or diC₁₋₆alkylamino;

- 5 6. A compound of formula (IA) according to any one of Claims 3 to 5 in which:

R¹ is methyl;

Q is -(CH₂)_n- where n is 3 to 5, more preferably 3 or 4;

-CH₂CH=CHCH₂-;

- 10 -(CH₂)_pX(CH₂)_q- where p is 2 or 3, q is 1 or 2 and X is S(O)_x where x is 0, 1 or 2, more preferably 0, 0 or NR² where R² is H or C₁₋₆alkyl, more preferably C₁₋₃alkyl; or

-(CH₂)_rA(CH₂)_s- where r is 1 or 2, s is 1 or 2 and A is a cyclobutyl, phenyl or pyridyl;

15

with the proviso that the group $\begin{array}{c} \text{NH}_2 \\ | \\ -\text{CH}-\text{CO}_2\text{H} \end{array}$ has the natural L or (S) chirality; and pharmaceutically acceptable salts, esters and amides thereof.

20

7. A compound of formula (IA) according to any one of Claims 3 to 6 in which:

R¹ is methyl;

Q represents -(CH₂)_n- where n is 3 or 4;

25

-CH₂CH=CHCH₂-;

-(CH₂)₂SCH₂-; or

-CH₂ACH₂- where A is cyclopropyl;

and pharmaceutically acceptable salts, esters and amides thereof.

30

8. A compound of formula (IA)



- and salts, and pharmaceutically acceptable esters and amides thereof, in which:

35

R¹ is a C₁₋₆ straight or branched chain alkyl group, a

C_{2-6} alkenyl group, a C_{2-6} alkynyl group, a C_{3-6} cycloalkyl group or a C_{3-6} cycloalkyl C_{1-6} alkyl group;

Q is an alkylene, alkenylene or alkynylene group having 3 to 6 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl groups;

a group of formula $-(CH_2)_pX(CH_2)_q-$ where p is 2 or 3, q is 1 or 2 and X is $S(O)_x$ where x is 0, 1 or 2, 0 or NR^2 where R^2 is H or C_{1-6} alkyl; or

a group of formula $-(CH_2)_rA(CH_2)_s-$ where r is 0, 1 or 2, s is 0, 1 or 2 and A is a 3 to 6 membered carbocyclic or heterocyclic ring which may optionally be substituted by one or more suitable substituents with the proviso that Q is not $CH_2CH_2CH_2$ when R^1 is methyl; for use in therapy.

15

9. A compound selected from:

(S)- N^5 -(1-iminoethyl)ornithine;

(S)- N^6 -(1-iminoethyl)lysine;

(±)-E-2-amino-6-(1-iminoethylamino)-hex-4-enoic acid;

20

and

(S)-S-2-(1-iminoethylamino)ethylcysteine

and pharmaceutically acceptable salts, esters and amides thereof.

25

10. A pharmaceutical formulation comprising a compound of formula (I) or (IA) as defined according to any one of Claims 1 to 7 or 9 or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients.

30

11. A method for the preparation of a compound of formula (IA) according to any one of Claims 3 to 7 which comprises the reaction of an amino acid of formula (II):

35



or a protected derivative thereof, with a compound of formula (III):



- 5 wherein L is a leaving group and R¹ and Q are as defined in any one of Claims 3 to 7, followed by removal of any protecting groups present.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/02387

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C07C257/14; A61K31/155; C07C257/16; C07C323/58		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07C	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	GB,A,2 240 041 (SOCIÉTÉ DE CONSEILS DE RECHERCHE ET D' APPLICATION SCIENTIFIQUES) 24 July 1991 cited in the application see claims 1,3,5,7	1,9,10
X	CHEMICAL ABSTRACTS, vol. 114, no. 9, 4 March 1991, Columbus, Ohio, US; abstract no. 79367d, D. D. REES 'Characterization of three inhibitors of endothelium nitric oxide synthase in vitro and in vivo' page 500 ; see abstract & BR. J. PHARMACOL. vol. 101, no. 3, 1990, pages 746 - 752 cited in the application	1,9
-/-		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> ¹⁰ Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </div> <div style="width: 45%;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">25 MARCH 1993</div>		Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">14. 04. 93</div>
International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div>		Signature of Authorized Officer <div style="text-align: center; font-weight: bold;">SEUFERT G.H.</div>

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	CHEMICAL ABSTRACTS, vol. 82, no. 1, 6 January 1975, Columbus, Ohio, US; abstract no. 1740w, V. B. PLAPP ET AL. 'Determination of epsilon-acetimidyllysine in proteins' page 162 ; see abstract & ANAL. BIOCHEM. vol. 62, no. 1, 1974, pages 291 - 294 & Chem. Subst. Index 1972-76, vol. 76-85, 21836CS	9
A	WO,A,9 104 024 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 4 April 1991 cited in the application see claims	1,3,10
A	EP,A,0 446 699 (MERRELL DOW PHARMACEUTICALS) 18 September 1991 cited in the application see claims	1,3,10
A	CHEMICAL ABSTRACTS, vol. 111, no. 15, 9 October 1989, Columbus, Ohio, US; abstract no. 130173e, A. PROUDFOOT ET AL. 'Conformation-directed recombination of enzyme-activated peptide fragments: a simple and efficient means to protein engineering' page 385 ; see abstract & J. BIOL. CHEM. vol. 264, no. 15, 1989, pages 8764 - 8770 & Chem. Subst. Index 1972-76, vol. 76-85, 52012CS	9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB92/02387

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
"Remark: Although claim 2 is directed to a method of treatment of the human
/animal body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9202387
SA 68539

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 25/03/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-2240041	24-07-91	AU-B- 629777	08-10-92
		AU-A- 6837690	27-06-91
		DE-A- 4041283	27-06-91
		FR-A- 2656220	28-06-91
		JP-A- 4364124	16-12-92
		LU-A- 87867	07-05-91
		NL-A- 9002720	16-07-91
		SE-A- 9003974	23-06-91
		US-A- 5081148	14-01-92
WO-A-9104024	04-04-91	US-A- 5028627	02-07-91
		AU-A- 6409690	18-04-91
		CA-A- 2065040	14-03-91
		JP-T- 5500219	21-01-93
EP-A-0446699	18-09-91	JP-A- 4270255	25-09-92

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINE(S) OR MARK(S) ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.